J. Plant Biol. (2011) 54:92–100
DOI 10.1007/s12374-011-9147-x

ORIGINAL RESEARCH

Ammonium-Stimulated Root Hair Branching is Enhanced by Methyl Jasmonate and Suppressed by Ethylene in *Arabidopsis thaliana*

Na Yang · Changhua Zhu · Lijun Gan · Denny Ng · Kai Xia

Received: 9 November 2010 / Revised: 13 January 2011 / Accepted: 14 January 2011 / Published online: 27 January 2011

Abstract Root hair development is orchestrated by nutritional factors and plant hormones. We investigated the action of ammonium (NH_4^+) and its interactions with methyl jasmonate (MeJA) and ethylene in *Arabidopsis* root hair growth. The formation of root hair branches was dramatically stimulated in media containing 1.25 to 20 mM NH_4^+ at pH values of 4.0 to 6.5. The NH_4^+-treated root hairs showed a very short tip growth stage and swells on the sides that indicated the emergence of branches. MeJA (0.08 to 10 μM) worked in synergism with NH_4^+ to enhance hair branching. In contrast, ethylene had an antagonistic effect; the stimulation of hair branching by NH_4^+ was suppressed by the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) and was diminished in ethylene-overproducing mutant *eto1-1* seedlings. Moreover, the application of Ag^+, an ethylene inhibitor, reduced the ACC-induced inhibition of NH_4^+-stimulated hair branching and restored NH_4^+-stimulated hair branching in *eto1-1* seedlings. Thus, the actions of jasmonate and ethylene appear to be dependent on nutritional conditions such as available nitrogen.

Keywords *Arabidopsis* · Ammonium · Methyl jasmonate · Ethylene · Root hair

Na Yang and Changhua Zhu equally contributed to this article.

N. Yang · C. Zhu · L. Gan · K. Xia (✉)
College of Life Sciences, Nanjing Agricultural University,
Nanjing 210095, China
e-mail: zhish@njau.edu.cn

D. Ng
NCA Biotech, Inc.,
3406 Pomona Blvd.,
Pomona, CA 91768, USA

Abbreviations

ACC 1	Aminocyclopropane-1-carboxylic acid
AVG	Aminoethoxyvinylglycine
AOA	Aminooxyacetic acid
IAA	Indole-3 acetic acid
JA	Jasmonic acid
MeJA	Methyl jasmonate
ROS	Reactive oxygen species

Introduction

Root hairs originate from the polar outgrowth of specific root epidermal cells, called trichoblasts, and greatly increase root surface area and absorption of nutrient ions and water (Carol and Dolan 2006). During root hair morphogenesis, a bulge is initially formed on the outer surface of a trichoblast at the distal end, and through highly polarized cell expansion, it protrudes perpendicular to the root surface, resulting in a thin cylindrical structure (Gilroy and Jones 2000; Ryan et al. 2001). Root hair development is modulated by a number of cellular processes, including cytoskeletal dynamics, tip-focused cytoplasmic calcium gradients, and vesicle/membrane trafficking (Galway et al. 1997; Carol and Dolan 2002). Reactive oxygen species are also signals in the regulation of root hair tip growth (Foreman et al. 2003; Carol et al. 2005).

Root hair formation is influenced by plant hormone-based signaling pathways, especially ethylene and auxin pathways (Pitts et al. 1998; Rahaman et al. 2002). The ethylene response mutant *ctr1* possesses ectopic root hairs on its atrichoblasts (Dolan et al. 1994). This is consistent with the finding that in wild-type *Arabidopsis*, the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC)

can induce ectopic root hair formation (Tanimoto et al. 1995; Masucci and Schiefelbein 1996; Pitts et al. 1998), whereas the ethylene biosynthesis inhibitor aminoethoxyvinylglycine (AVG) and the ethylene action inhibitor Ag^+ can reduce root hair formation (Masucci and Schiefelbein 1994; Tanimoto et al. 1995). Auxin levels in the trichoblast also strongly influence root hair growth. Trichoblast-specific overexpression of the auxin efflux transporters PINOID, PIN3, PIN2, PIN4, and PGP4 results in increased auxin efflux from these cells, and the reduced cellular auxin level inhibits root hair growth (Lee and Cho 2006; Cho et al. 2007). Jasmonates have been reported to stimulate root hair formation (Zhu et al. 2006). Both jasmonic acid (JA) and methyl jasmonate (MeJA) at 1 mM stimulated root hair formation by up to 2.5- and 4-fold, respectively, and jasmonates were shown to act synergistically with ethylene to induce root hair formation.

Many mineral nutrients, including phosphate, iron, manganese, and zinc, have been shown to strongly influence root hair initiation and elongation, which would in turn directly or indirectly affect nutrient uptake by roots (Peterson and Stevens 2000). Under conditions of low nutrient availability, root hair density and length increase. Phosphate has the strongest and best-characterized effects on root hairs (Bates and Lynch 1996). In *Arabidopsis*, the root hair density on roots grown in low-phosphorous conditions (1 mmol m^{-3}) was 5-fold the density on roots grown in high-phosphorous conditions (1,000 mmol m^{-3}), and root hair density decreased logarithmically in response to increasing phosphorus concentrations within that range (Ma et al. 2001). Iron-deficient roots produced ectopic hairs, and hair length doubled (Schmidt et al. 2000). The combined effects of mineral ions and plant hormones on root hair development have been studied. The auxin indole-3-acetic acid (IAA), the auxin transport inhibitor 2-(*p*-chlorophenoxy)-2-methylpropionic acid (CMPA), the ethylene precursor ACC, and the ethylene synthesis inhibitor aminooxyacetic acid (AOA) all increased root hair density in high-phosphorus conditions, but had very little effect in low-phosphorus conditions, suggesting that low phosphorus does not act via ethylene or IAA (Ma et al. 2001). Ethylene levels are enhanced in the presence of both Fe and P, suggesting similar transduction pathways (Lynch 1998; Romera et al. 1999).

Nitrogen is a major limiting nutrient for many plants. Plant roots take up soil nitrogen primarily as nitrate (NO_3^-) and ammonium (NH_4^+) (Forde 2002). Nitrogen initiates many physiological and morphological responses in roots. A striking example is the effect of nitrate availability on lateral roots. When *Arabidopsis* roots were exposed to a locally concentrated supply of NO_3^-, there was a localized 2-fold increase in the mean rate of lateral root elongation; however, high rates of NO_3^- supply to the roots had a systemic inhibitory effect on lateral root development (Zhang and Forde 1998; Zhang et al. 1999). In two grass species, *Deschampsia flexuosa* (L.) Trin. and *Poa annua* L., root hair growth was responsive to low nitrogen availability, and the response was generally greater to NH_4^+ than to NO_3^- (Robinson and Rorison 1987). Both NH_4^+ and NO_3^- were reported to interact with ROP signaling, which modulates root hair tip growth (Bloch et al. 2010).

In the present study, we investigated the action of NH_4^+ and its interactions with methyl jasmonate (MeJA) and ethylene in *Arabidopsis* root hair growth. We show that NH_4^+ stimulates the formation of root hair branches and inhibits root hair elongation in *Arabidopsis*. Moreover, the stimulation by NH_4^+ was enhanced by MeJA and diminished by ethylene.

Materials and Methods

Plant Materials and Growth Conditions

Seeds of wild-type *Arabidopsis* (ecotype Columbia) and an ethylene-overproducing mutant *eto1-1* were kept under dry conditions at 4°C until use.

The culture medium contained 5 mM KNO_3, 2 mM $MgSO_4$, 2 mM $CaSO_4$, 2.5 mM KH_2PO_4, 70 μM H_3BO_3, 14 μM $MnCl_2$, 1 μM $ZnSO_4$, 0.5 μM $CuSO_4$, 10 μM NaCl, 0.2 μM Na_2MoO_4, and 40 μM FeEDTA, and was solidified with 1% (w/v) agar. Succinic acid (Suc; 43 mM) and 2-(*N*-morpholino)-ethanesulfonic acid (Mes; 4.7 mM) were included, and the pH was adjusted to 5.5. The seeds were surface sterilized by immersion in 5% (v/v) NaOCl for 5 min and 96% (v/v) ethanol for 30 s, followed by four rinses in sterile water. The sterilized seeds were placed onto Petri dishes containing culture medium and kept at 4°C in the dark for 3 days. The dishes were transferred to a growth chamber and grown at 23°C in continuous light.

To test the effect of nitrogen, KNO_3 and NH_4Cl were used to supply NO_3^- and NH_4^+, respectively. NH_4NO_3 was applied to supply both NO_3^- and NH_4^+. In addition, KCl was added to compensate for K^+ in the applied KNO_3 and for Cl^- in the applied NH_4Cl. These four chemicals were added to the culture medium at the indicated concentrations.

Application of Plant Hormones and Inhibitors

MeJA (Wako Pure Chemical Industries) was dissolved in a small amount of ethanol and then diluted with distilled water to a 1 mM stock concentration. The ethylene precursor ACC (Sigma) and the ethylene action inhibitor $AgNO_3$ were each dissolved in distilled water to make a 1 mM stock solution. The required amounts of the stock solutions were mixed with agar at a temperature of 45–50°C.

Counting the Branched Root Hairs

Arabidopsis seedlings were transferred to a microscope slide with a thin layer of Murashige and Skoog medium containing 3% Suc and 1% agarose. Root hairs were viewed using differential interference optics microscopy (AXION IMAGE-A1; Zeiss, Germany). During observation, the slides were incubated in a humid environment at room temperature in the dark. Photographs taken using a digital camera (Nikon) and were used to determine the branched root hairs as a percentage of the total root hairs.

Measurement of Root Hair Length

Seedlings grown in Petri dishes were placed on the stage of a stereomicroscope (MZFLIII; Leica Microsystem, Wetzlar, Germany), and the apical segment within 1 cm of the apex was photographed for each of 20 seedlings. Root hair lengths were calculated by analyzing the digital images with Motic Images Plus 2.0 (China Group Co., Ltd). Statistical significance of the differences between mean values was determined using SPSS.

Results

Ammonium Stimulation of Root Hair Branching and Inhibition of Root Hair Elongation

Four-day-old *Arabidopsis* seedlings were grown on medium containing 10 or 20 mM NH_4NO_3, KNO_3, NH_4Cl, or KCl, respectively, on vertically oriented agar plates and examined for root hair formation (Table 1). On fresh agar medium, the seedlings formed only a few branched hairs (2.3–3.0%). With 10 and 20 mM KNO_3, there was a very slight increase in the percentage of branched hairs (3.9% and 4.5%, respectively), whereas supplementation with NH_4Cl or NH_4NO_3 greatly increased the percentage of branched hairs. At both 10 and 20 mM, NH_4Cl induced about 16.0% branched root hairs, and NH_4NO_3 at 10 and 20 mM induced 14.8% and 25.2%, respectively. KCl at 10 and 20 mM showed no effect on branching, thus eliminating K^+ and Cl^- as the regulators of root hair branching. Therefore, NH_4^+ acted to strongly stimulate the formation of branched root hairs, whereas NO_3^- had very little effect.

In addition to root hair branching, ammonium also tightly controlled the elongation of root hairs. At the indicated concentrations, NH_4Cl and NH_4NO_3 caused decreases of 28% and 37%, respectively, in root hair length. In contrast, KNO_3 and KCl at the tested concentrations showed little effect on root hair length. These results suggest that NH_4^+ was responsible for the pronounced inhibition of root hair elongation.

Dose Dependency of the Effects of Ammonium on Root Hairs

To further examine the effect of NH_4^+ on root hair development, 4-day-old wild-type *Arabidopsis* seedlings were transferred to agar medium supplemented with NH_4NO_3 at a range of concentrations. Both root hair branching and elongation responded to NH_4^+ in a dose-dependent manner. Concentrations of 1.25 to 20 mM NH_4^+ promoted root hair branching, with 1.25, 5, and 20 mM NH_4^+ producing 3-, 4-, and 10-fold increases in branched hairs, respectively (Fig. 1a). In addition, the inhibitory effects of 1.25, 5, and 20 mM NH_4^+ on root hair elongation decreased root hair length by 18%, 27%, and 40%, respectively (Fig. 1b).

Morphological observations of roots grown in 20 mM NH_4NO_3 are shown in Fig. 1c–h. There were few branched hairs in the control roots without NH_4^+ (Fig. 1c), whereas

Table 1 Effect of nitrogen source on the induction of root hair branching in *Arabidopsis* seedlings

Nitrogen sources	Concentrations (mM)	Branched hair numbers	Branched hair percentages (%)	Root hair lengths (mm)
KNO_3	0	3.6	3.0±0.7	0.56±0.03
	10	4.7	3.9±0.8	0.53±0.02
	20	5.3	4.5±0.7	0.57±0.03
NH_4Cl	0	3.5	2.4±0.6	0.55±0.08
	10	24.9	16.2±0.1	0.38±0.04
	20	29.3	15.9±0.1	0.35±0.07
NH_4NO_3	0	3.5	2.4±0.6	0.55±0.08
	10	25.8	14.8±2.8	0.40±0.03
	20	48.4	25.2±0.4	0.35±0.03
KCl	0	3.6	3.0±0.7	0.56±0.03
	10	3.9	3.1±0.3	0.56±0.03
	20	3.4	3.0±0.4	0.58±0.04

Seedlings were grown for 4 days in culture medium and then transferred to medium supplemented with KNO_3, NH_4Cl, NH_4NO_3, or KCl. The numbers and lengths of root hairs branched within 1 cm of the root tip were determined 48 h after treatment. The values represent the means of 20 seedlings. Standard error (SE) is shown for the branched hair percentage (%) and root hair length

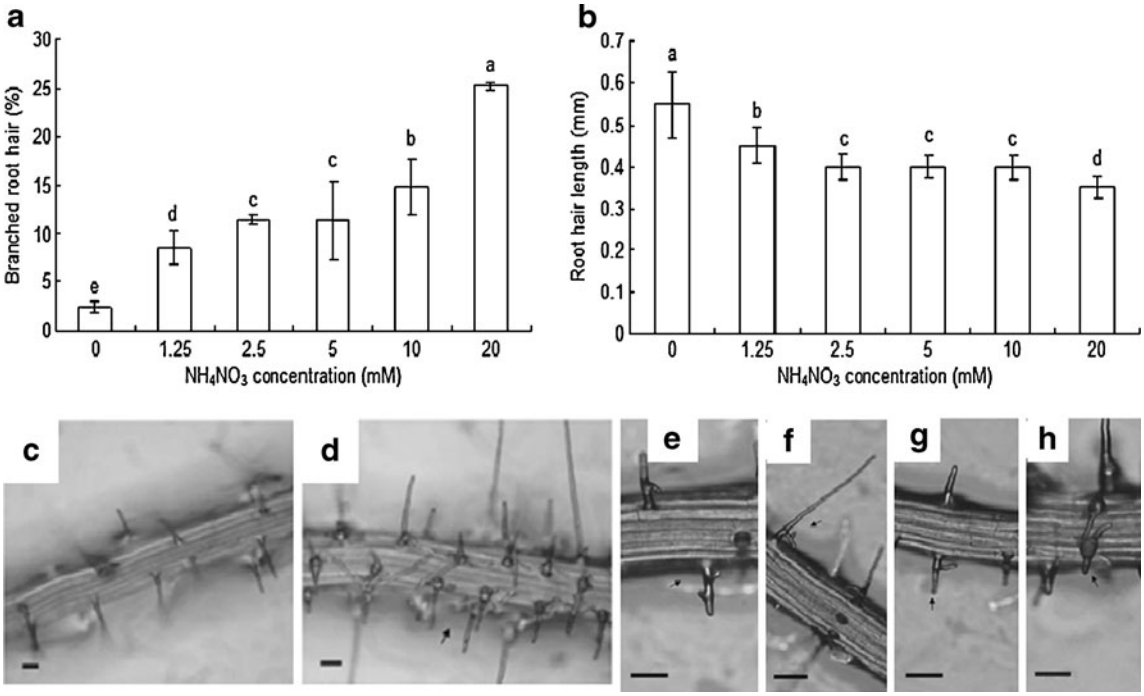

Fig. 1 Effects of NH_4NO_3 on the promotion of root hair branching. *Arabidopsis* seedlings were grown for 4 days in culture medium and then transferred to medium supplemented with NH_4NO_3 at the indicated concentrations. The **a** number of branched root hairs and **b** root hair length were determined 48 h after treatment. Values are the means of 20 seedlings. *Bars* indicate the standard error (SE). Values followed by different letters are significantly different at $P<0.05$. The photographs show the morphology of **c** a control root without NH_4NO_3 treatment and **d**–**h** roots treated with 20 mM NH_4NO_3. *Arrows* indicate branched root hairs. *Bar*=50 μm

many hairs were branched in the roots treated with NH_4^+ (Fig. 1d). The NH_4^+-treated roots exhibited one or two branches at the middle and base sides of growing hair cells (Fig. 1e, g), a secondary branch off of an initial branch (Fig. 1f), or several branches coming from a large bulge of epidermal cells (Fig. 1h).

As previous studies on lettuce suggested an effect of pH on root hair formation (Inoue et al. 2000), we tested whether the NH_4^+-stimulated formation of root hair branches occurred via a change of pH in the NH_4^+-supplemented medium. As shown in Fig. 2, in media with pH values ranging from 4.0 to 6.0, KNO_3 always induced the rate of branching to as low as 2–10%, whereas NH_4NO_3 strongly stimulated root hair branching. At pH 4.5 to 5.5, NH_4NO_3 induced 35–40% branched root hairs. Even at pH 4.0, NH_4NO_3 caused 20% branched root hair. Only at pH 6.0 did NH_4NO_3 show reduced induction of root hair branching. These data suggest that NH_4^+ stimulates root hair branching by a direct action, rather than indirectly via a pH change.

Effect of Ammonium on Cytoplasmic Changes Related to Root Hair Branching at the Root Tip

Growing root hairs display a polarized cytoplasmic organization in which organelles necessary for the formation of a new cell wall accumulate in the tip. Thus, the apical region of a growing hair cell, known as the clear zone, lacks large organelles and vacuoles (Ovecka et al. 2005). When hair tip growth is terminated, the polarized organization of the cytoplasm gradually disappears, large organelles and

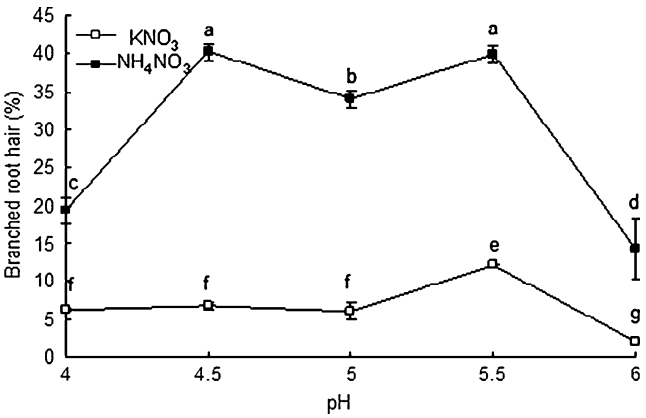

Fig. 2 Effects of NO_3^- and NH_4^+ on root hair branching in media with pH values ranging from 4 to 6. *Arabidopsis* seedlings were grown for 4 days in culture medium and then transferred to medium supplemented with 20 mM NH_4NO_3 or 20 mM KNO_3. The pH of the medium was adjusted to 4.0, 4.5, 5.0, 5.5, or 6.0. The number of branched root hairs was determined 48 h after treatment. Values are the means of 20 seedlings. *Bars* indicate the standard error (SE). Values followed by different letters are significantly different at $P<0.05$

vacuoles invade the tip, and the root hair becomes surrounded by only a thin cytoplasmic layer. The cytoarchitectural changes in root hairs during development in the absence and presence of NH_4^+ are shown in Fig. 3. In the control root hair without NH_4^+ treatment, increased cytoplasmic density was observed at the emerging tip in the bulge stage (Fig. 3a). The amount of dense cytoplasm increased, and a reverse-fountain type cytoplasmic streaming formed at the vigorous tip growth stage (Fig. 3b). The volume of dense cytoplasm and the rate of tip growth began to decrease in the hair cell before the growth termination stage (Fig. 3c). Finally, a thin layer of cytoplasm with a circulation-type movement of organelles was observed surrounding a large vacuole in the fully grown hair (Fig. 3d). In the NH_4^+-treated root hair, the cytoarchitecture of the outgrowth resembled that of the control hair at the bulge stage and early tip growth stage (Fig. 3e). However, the tip of the NH_4^+-treated root hair had a very short fast-growth stage (Fig. 3f), was soon invaded by a large vacuole, and lacked vigorous cytoplasmic streaming, except for small granules moving along the plasma membrane (Fig. 3g). Then, a swelling formed in the side of the hair, from which a branch finally emerged (Fig. 3h).

MeJA Enhancement of NH_4^+-Stimulated Branched Root Hair Formation

Our previous work suggested that jasmonates promoted the formation of root hairs, including branched hairs in *Arabidopsis* (Zhu et al. 2006); therefore, we tested the possible involvement of jasmonates in NH_4^+-stimulated branch formation. MeJA strongly enhanced NH_4^+-stimulated root hair branching, showing a synergistic effect with NH_4^+ (Fig. 4).

At concentrations of 0.08 to 10 μM, MeJA caused only a small increase in the percentage of branched root hairs (2–6%) in medium without NH_4NO_3 (Fig. 4a). However, MeJA dose-dependently promoted branched hair formation in the presence of 20 mM NH_4NO_3. Treatment with NH_4NO_3 alone caused the formation of about 20% branched hairs; this was increased to about 27%, 30%, 31%, and 36% with 0.08, 0.4, 2, and 10 mM MeJA, respectively. Moreover, MeJA worked in synergism with NH_4^+, rather than with NO_3^-, in promoting root hair branching (Fig. 4b). At 2 μM, MeJA produced little increase in the percentage of branched root hairs in control medium or medium with 20 mM KNO_3. However, in medium supplemented with 20 mM NH_4NO_3 or NH_4Cl, MeJA at 2 μM resulted in a 61.6% or 137.1% increase in branched hairs, respectively.

Ethylene Inhibition of NH_4^+-Stimulated Branched Root Hair Formation

Ethylene, a positive regulator of root hair development (Tanimoto et al. 1995), may be intimately linked to the regulation of root hair development at all levels, from cell-fate specification to tip growth. We tested the involvement of ethylene in NH_4^+-stimulated branched hair formation. In contrast to MeJA, ethylene, applied as the ethylene precursor ACC, antagonized the effect of NH_4^+ by dramatically inhibiting NH_4^+-stimulated root hair branching (Fig. 5).

Treatment with 20 mM NH_4NO_3 alone caused 24% hair branching, and this was reduced to 5% by 0.04 μM ACC (Fig. 5a). At 0.2 μM and higher, ACC almost completely blocked the action of NH_4^+. The ethylene-overproducing mutant *eto1-1* did not form root hair branches in response to NH_4^+ (Fig. 5b). Although 20 mM NH_4NO_3 increased branched root hair formation approximately 10-fold in *Arabidopsis* wild-type seedlings, it had little effect in *eto1-1* seedlings. In addition, the application of Ag^+, an ethylene action inhibitor, relieved the ACC inhibition of NH_4^+-stimulated root hair branching in wild-type *Arabidopsis* (Fig. 5c) and recovered branched root hair formation in *eto1-1* seedlings supplied with NH_4^+ (Fig. 5d).

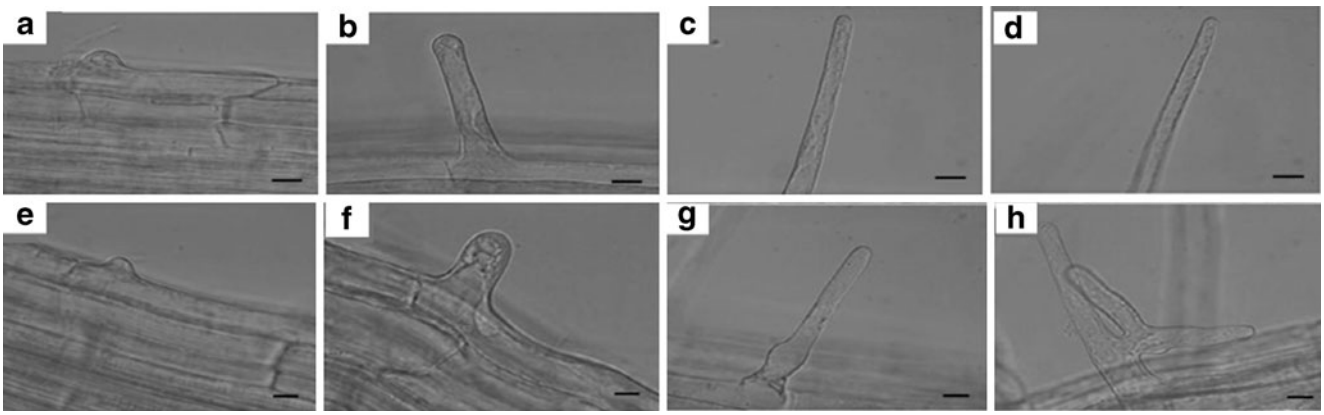

Fig. 3 Cytoarchitecture of root hair growth with and without NH_4^+. *Arabidopsis* seedlings were grown for 4 days in culture medium and then transferred to **a**–**d** control medium without a supply of NH_4NO_3 or **e**–**h** medium supplemented with 20 mM NH_4NO_3. *Bar*=10 μm

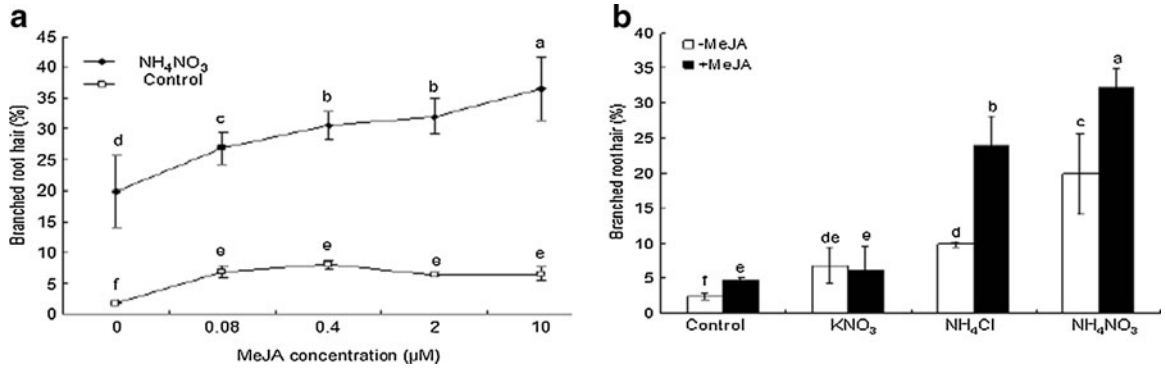

Fig. 4 The synergistic effect of MeJA on NH_4^+-promoted root hair branching. *Arabidopsis* seedlings were grown for 4 days in culture medium and then transferred to **a** control medium (identical to culture medium) and medium supplemented with 20 mM NH_4NO_3 in the presence of MeJA at the indicated concentrations, or **b** control medium (identical to culture medium) and medium supplemented with 20 mM KNO_3, NH_4Cl, or NH_4NO_3 in the presence (+*MeJA*) and absence (−*MeJA*) of 2 μM MeJA. The branched root hair percentage was determined after 48 h of treatment. Values are the means of 20 seedlings. *Bars* indicate the standard error (SE). Values followed by different letters are significantly different at $P<0.05$

Discussion

Physiological Cues Related to Root Hair Branching

Arabidopsis root hair development includes several phases: epidermal cell fate specification, initiation, subsequent tip growth, and maturation (Dolan et al. 1994; Gilroy and Jones 2000). Root hair branching has been observed, by others and us, mainly during the tip growth phase. In the growing hair tip, the polarized targeting and fusion of Golgi vesicles bring about the exocytosis of new plasma membrane and cell wall components, leading to an

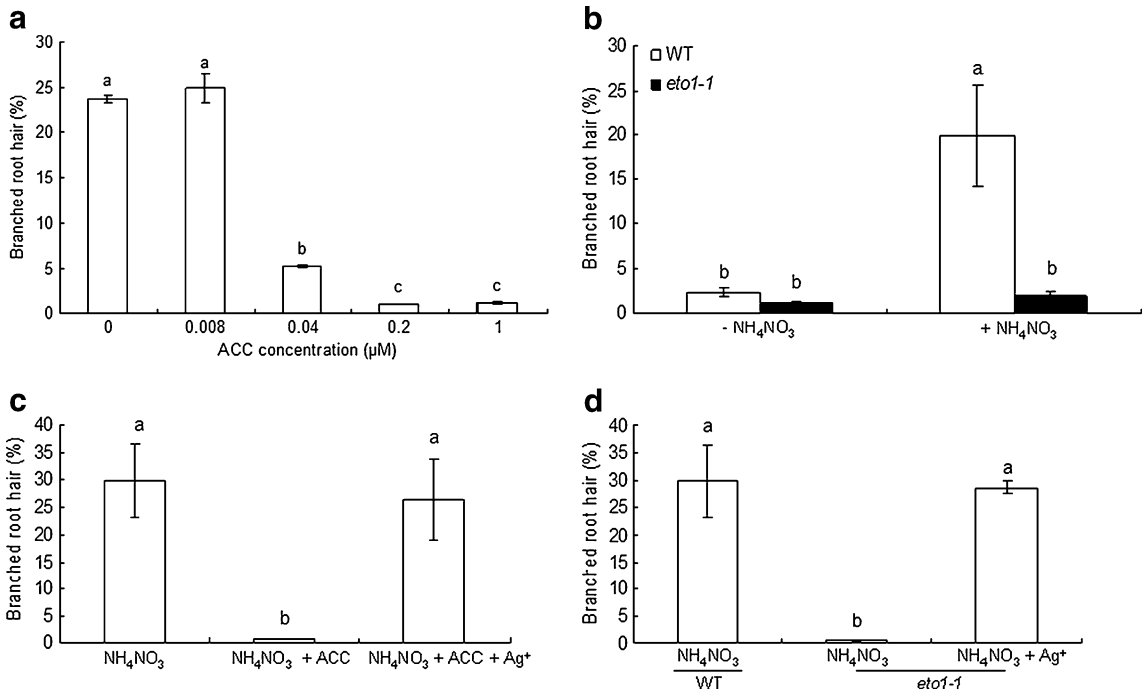

Fig. 5 Antagonistic effect of ethylene on NH_4^+-promoted root hair branching. Seedlings of wild-type *Arabidopsis* and the ethylene-overproducing mutant *eto1-1* were grown for 4 days in culture medium and then transferred to treatment media. The number of branched root hairs was determined 48 h after treatment. **a** Wild-type seedlings were treated with 20 mM NH_4NO_3 in the presence of ACC at the indicated concentrations. **b** Wild-type and *eto1-1* seedlings were treated with (+) and without (−) 20 mM NH_4NO_3. **c** Wild-type seedlings were treated with 20 mM NH_4NO_3, 20 mM NH_4NO_3 plus 1 μM ACC, and 20 mM NH_4NO_3 plus 1 μM ACC plus 2 μM Ag^+. **d** Wild-type seedlings were treated with 20 mM NH_4NO_3, and *eto1-1* seedlings were treated with 20 mM NH_4NO_3 in the absence and presence of 2 μM Ag^+. Values are the means of 20 seedlings. *Bars* indicate the standard error (SE). Values followed by different letters are significantly different at $P<0.05$

elongated hair-like morphology. Elongation ceases in mature root hairs, and the whole hair is filled with a large subapical vacuole, leaving only a thin layer of cytoplasm at the periphery of the cell (Ryan et al. 2001).

Hair tip growth involves both the actin cytoskeleton (Kandasamy et al. 2009) and microtubules (Bibikova et al. 1999). A high concentration of cytosolic Ca^{2+} in the hair tip, oscillation of the extracellular and intracellular pH, production of extracellular reactive oxygen species (ROS), and signaling via phospholipids can regulate of hair tip growth and polarity (Libault et al. 2010). *Arabidopsis* Rop2 GTPase has been proposed to act as a positive regulatory switch for tip growth (Jones et al. 2002). The concentration of phosphatidylinositols is also likely to regulate root hair elongation (Kusano et al. 2008; Thole et al. 2008).

Wild-type plants form few branched hairs, while mutants such as *tip1*, *cow1*, *cen2*, *cen3*, and *scn1* exhibit a greater number of branched hairs (Schiefelbein et al. 1993; Grierson et al. 1997, 2001; Ryan et al. 1998). Hair branching can also be caused by actin and microtubule antagonists and by drugs that disrupt myosin ATPases or exocytosis (Bibikova et al. 1999; Ovecka et al. 2000). In Fe deficiency, about one third of the hairs are branched in *Arabidopsis* plants (Müller and Schmidt 2004), suggesting that nutritional signals influence hair tip growth. The formation of root hair branches may be determined by a developmental program for increasing the absorptive surface of Fe-deficient plants.

Here, we propose a novel effect of NH_4^+ in stimulating root hair branching. NH_4^+ may disturb vesicle trafficking for the establishment of polarity in hair tips. We showed that NH_4^+-treated root hairs had a short fast-growth stage, were quickly invaded by a large vacuole, and lacked vigorous cytoplasmic streaming in the tip (Fig. 3). Little is known about the actions of NH_4^+ on the cytoskeleton, the cytosolic Ca^{2+} concentration, and other physiological changes during hair tip growth. These subjects deserve further research attention.

Stimulation of Root Hair Branching as a Response to NH_4^+ Toxicity or NH_4^+-Induced Stress Signaling

NH_4^+ is the predominant nitrogen source in many natural and agricultural ecosystems (Vitousek et al. 1982). For example, NH_4^+ concentrations range from 0.4 to 4.0 M in solutions of forest floor soil (Bijlsma et al. 2000) and from 2.0 to 20 M in agricultural soils (Britto and Kronzucker 2002). NH_4^+ can be toxic to many, if not all, plants cultured with NH_4^+ as the exclusive nitrogen source. Obvious symptoms of NH_4^+ toxicity consist of leaf chlorosis, growth suppression, decreased root/shoot ratio, and reduced mycorrhizal associations. Physiological changes in NH_4^+-fed plants include a decline in tissue levels of cations such as K^+ and Mg^{2+}, an increase of inorganic anions such as chloride and sulfate, cytosolic pH disturbances, shifts in plant carbohydrate status, uncoupling of photophosphorylation, and alterations in hormonal balances (Britto and Kronzucker 2001).

Root growth and development are closely related to the nitrogen concentration in the external medium. Low and high concentrations of NO_3^- respectively stimulate and inhibit growth (Zhang et al. 1999, Zhang and Forde 2000; Forde 2002). Nitrogen availability was also reported to have a remarkable effect on root hair growth (Robinson and Rorison 1987; Bloch et al. 2010). We found that at concentrations ranging from 1.25 to 20 mM, NH_4^+ strongly stimulated root hair branching, although at these concentrations, NH_4^+ may also result in toxicity in NH_4^+-sensitive plants. Therefore, it is plausible that NH_4^+-stimulated formation of root hair branches in *Arabidopsis* is a morphological response to NH_4^+ toxicity or NH_4^+-induced stress signals.

The phenotype of branched root hairs was also found in the *Arabidopsis supercentipedel* (*scn1*) mutant. The *scn1* produces multiple hairs from one initiation site (Parker et al. 2000), and the root hairs are large outgrowths with multiple irregular bulges. In a wild-type hair, ROS are usually focused at a single point at the growing hair tip; however, a *scn1* hair has multiple foci of ROS in a cell with several bulges (Carol et al. 2005). We also found that diphenylene iodonium chloride (DPI), an inhibitor of NADPH oxidase, blocked NH_4^+-stimulated hair branching (unpublished data). Therefore, it is suggested that ROS may be an NH_4^+-induced stress signal leading to the formation of hair branches. A similar suggestion came from a recent report on ROP GTPases and their regulatory proteins in root hair development. Root hair swelling in constitutively active ROP11 mutant plants (*Atrop11CA*) was strongly dependent on the growth medium composition, particularly the presence of NH_4^+, and depolarized growth of root hairs in *Atrop11CA* plants was associated with the abolishment of the tip-focused ROS gradient (Bloch et al. 2010).

Relationship Between Jasmonates and Ethylene in the Control of Root Hair Growth

Jasmonates and ethylene control a variety of developmental processes in plants. Jasmonates are necessary for pollen development and anther dehiscence, inhibition of seed germination and root growth, response to mechanical wounding and abiotic stresses, and defense against pests and pathogens (Pauwels et al. 2009). Ethylene regulates fruit ripening, programmed cell death, and responsiveness to stress and pathogens. It also triggers the triple response of etiolated seedlings and induces prolific root hair formation (Guo and Ecker 2004).

Jasmonates and ethylene have been reported to interact both positively and negatively with each other. The biosynthesis of both jasmonate and ethylene is triggered by pests, pathogens, and wounding. The two hormones synergistically induce the expression of defense-related genes such as PATHOGENESIS-RELATED5 (PR5), PLANT DEFENSIN1.2 (PDF1.2), a chitinase (CHI-B), a hevein-like (HEL) protein, and proteinase inhibitors (PIN) (Xu et al. 1994; Penninckx et al. 1998; Norman-Setterblad et al. 2000; Ellis and Turner 2001). They simultaneously activate ETHYLENE TRANSCRIPTION FACTOR1 (ERF1), a transcription factor mediating defense responses against pathogens (Lorenzo et al. 2003). ORA59 is an essential integrator of the jasmonate and ethylene signal transduction pathways (Pre et al. 2008). The jasmonate receptor COI1 is involved in ethylene-induced inhibition of *Arabidopsis* root growth in the light (Adams and Turner 2010). In contrast, ethylene inhibits the expression of some jasmonate-responsive genes encoding vegetative storage proteins (VSPs) and a thionin (Thi1.2) (Rojo et al. 1999; Norman-Setterblad et al. 2000; Ellis and Turner 2001), and jasmonates suppress ethylene-induced hypocotyl hook formation in the triple response in a *COI1*-dependent manner (Ellis and Turner 2002).

Our previous work revealed a pronounced effect of jasmonates on the promotion of *Arabidopsis* root hair formation. This effect was blocked by the ethylene inhibitors Ag^+ and AVG and was diminished in the ethylene-insensitive mutants *etr1-1* and *etr1-3*. Furthermore, the jasmonate biosynthesis inhibitors ibuprofen and salicylhydroxamic acid suppressed ethylene-induced root hair formation and decreased the root hairs in ethylene-overproducing *eto1-1* seedlings. Therefore, root hair formation in *Arabidopsis* may involve the concerted actions of jasmonates and ethylene (Zhu et al. 2006).

Here, we showed a reverse action between jasmonates and ethylene on NH_4^+-stimulated formation of root hair branches. MeJA enhanced and ethylene suppressed root hair branching in *Arabidopsis* supplied with NH_4^+. These results raise the possibility that the interaction between jasmonates and ethylene is dependent on certain environmental factors such as the presence of NH_4^+. Collectively, these findings shed light on the interactions between hormones and nitrogen supply during root hair growth.

Acknowledgment This work was supported by a grant from the National Natural Science Foundation of China (30871460).

References

Adams E, Turner J (2010) COI1, a jasmonate receptor, is involved in ethylene-induced inhibition of *Arabidopsis* root growth in the light. J Exp Bot. doi:10.1093/jxb/erq240

Bates TR, Lynch JP (1996) Stimulation of root hair elongation in *Arabidopsis thaliana* by low phosphorus availability. Plant Cell Environ 19:529–538

Bibikova TN, Blancaflor EB, Gilroy S (1999) Microtubules regulate tip growth and orientation in root hairs of *Arabidopsis thaliana*. Plant J 17:657–665

Bijlsma RJ, Lambers H, Kooijman SALM (2000) A dynamic whole plant model of integrated metabolism of nitrogen and carbon. 1. Comparative ecological implications of ammonium–nitrate interactions. Plant Soil 220:49–69

Bloch D, Monshausen G, Singer M, Gilroy S, Yalovsky S (2010) Nitrogen source interacts with ROP signalling in root hair tip-growth. Plant Cell Environ. doi:10.1111/j.1365-3040.2010.02227.x

Britto DT, Kronzucker HJ (2001) Constancy of nitrogen turnover kinetics in the plant cell: insights into the integration of subcellular N fluxes. Planta 213:175–181

Britto DT, Kronzucker HJ (2002) NH_4^+ toxicity in higher plants: a critical review. J Plant Physiol 159:567–584

Carol RJ, Dolan L (2002) Building a hair: tip growth in *Arabidopsis thaliana* root hairs. Phil Trans R Soc Lond 357:815–821

Carol RJ, Dolan L (2006) The role of reactive oxygen species in cell growth: lessons from root hairs. J Exp Bot 57:1829–1834

Carol RJ, Takeda S, Linstead P, Durrant MC, Kakesova H, Derbyshire P, Drea S, Zarsky V, Dolan L (2005) A RhoGDP dissociation inhibitor spatially regulates growth in root hair cell. Nature 438:1013–1016

Cho M, Lee SH, Cho HT (2007) P-glycoprotein4 displays auxin efflux transporter like action in *Arabidopsis* root hair cells and tobacco cells. Plant Cell 19:3930–3943

Dolan L, Duckett CM, Grierson C, Linstead P, Schneider K, Lawson E, Dean C, Poethig S, Roberts K (1994) Clonal relationships and cell patterning in the root epidermis of *Arabidopsis*. Development 120:2465–2474

Ellis C, Turner JG (2001) The *Arabidopsis* mutant *cev1* has constitutively active jasmonate and ethylene signal pathways and enhanced resistance to pathogens. Plant Cell 13:1025–1033

Ellis C, Turner JG (2002) A conditionally fertile *coi1* allele indicates cross-talk between plant hormone signalling pathways in *Arabidopsis thaliana* seeds and young seedlings. Planta 215:549–556

Forde BG (2002) Local and long-range signaling pathways regulation plant responses to nitrate. Annu Rev Plant Biol 53:203–224

Foreman J, Demidchik V, Bothwell JHF, Mylona P, Miedema H, Torres MA, Linstead P, Costa S, Brownlee C, Jones JDG, Davies JM, Dolan L (2003) Reactive oxygen species produced by NADPH oxidase regulate plant cell growth. Nature 422:442–446

Galway ME, Heckman JW, Schiefelbein JW (1997) Growth and ultrastructure of *Arabidopsis* root hairs: the *rhd3* mutation alters vacuole enlargement and tip growth. Planta 201:209–218

Gilroy S, Jones DL (2000) Through form to function: root hair development and nutrient uptake. Trends Plant Sci 5:56–60

Grierson CS, Roberts K, Feldmann KA, Dolan L (1997) The COW1 locus of *Arabidopsis* acts after RHD2, and in parallel with RHD3 and TIP1, to determine the shape, rate of elongation, and number of root hairs produced from each site of hair formation. Plant Physiol 115:981–990

Grierson CS, Parker JS, Kemp AC (2001) *Arabidopsis* genes with roles in root hair development. J Plant Nutr Soil Sci 164:131–140

Guo H, Ecker JR (2004) The ethylene signaling pathway: new insights. Curr Opin Plant Biol 7:40–49

Inoue Y, Yamaoka K, Kimura K, Sawai K, Arai T (2000) Effects of low pH on the induction of root hair formation in young lettuce (*Lactuca sativa* L. cv. Grand Rapids) seedlings. J Plant Res 113:39–44

Jones MA, Shen JJ, Fu Y, Li H, Yang Z, Grierson CS (2002) The *Arabidopsis* Rop2 GTPase is a positive regulator of both root hair initiation and tip growth. J Plant Res 14:763–776

Kandasamy MK, McKinney EC, Meagher RB (2009) A single vegetative actin isovariant overexpressed under the control of multiple regulatory sequences is sufficient for normal *Arabidopsis* development. Plant Cell 21:701–718

Kusano H, Testerink C, VermeerJEM TsugeT, Shimada H, Oka A, Munnik T, Aoyama T (2008) The *Arabidopsis* phosphatidylinositol phosphate 5-kinase PIP5K3 is a key regulator of root hair tip growth. Plant Cell 20:367–380

Lee SH, Cho HT (2006) PINOID positively regulates auxin efflux in *Arabidopsis* root hair cell and tobacco cells. Plant Cell 18:1604–1616

Libault M, Brechenmacher L, Cheng J, Xu D, Stacey G (2010) Root hair systems biology. Trends Plant Sci. doi:10.1016/j.tplants.2010.08.010

Lorenzo O, Piqueras R, Sanchez-Serrano JJ, Solano R (2003) ETHYLENE RESPONSE FACTOR1 integrates signals from ethylene and jasmonate pathways in plant defense. Plant Cell 15:165–178

Lynch JP (1998) Root architecture and phosphorus acquisition efficiency in common bean. In: Flores HE, Lynch JP, Eisenstat D (eds) Radical biology: advances and perspectives on the function of plant roots. American Society of Plant Physiologists, Rockville, pp 81–91

Ma Z, Bielenberg DG, Brown KM, Lynch JP (2001) Regulation of root hair density by phosphorus availability in *Arabidopsis thaliana*. Plant Cell Environ 29:459–467

Masucci JD, Schiefellbein JW (1994) The *rhd6* mutation of *Arabidopsis thaliana* alters root hair initiation through an auxin- and ethylene-associated process. Plant Physiol 106:1335–1346

Masucci JD, Schiefelbein JW (1996) Hormones act downstream of *TTG* and *GL2* to promote root hair outgrowth during epidermis development in the *Arabidopsis* root. Plant Cell 8:1505–1517

Müller M, Schmidt W (2004) Environmentally induced plasticity of root hair development in *Arabidopsis*. Plant Physiol 134:409–419

Norman-Setterblad C, Vidal S, Palva ET (2000) Interacting signal pathways control defense gene expression in *Arabidopsis* in response to cell wall-degrading enzymes from *Erwinia carotovora*. Mol Plant Microbe Interact 4:430–438

Ovecka M, Nadubinska M, Volkmann D, Baluska F (2000) Actomyosin and exocytosis inhibitors alter root hair morphology in *Poa annua*. Biologia 55:105–114

Ovecka M, Lang I, Baluska F, Ismail A, Illes P, Lichtscheidl IK (2005) Endocytosis and vesicle trafficking during tip growth of root hairs. Protoplasma 226:39–54

Penninckx IAMA, Thomma BPHJ, Buchala A, Metraux JP, Broekaert WF (1998) Concomitant activation of jasmonate and ethylene response pathways is required for induction of a plant defensin gene in *Arabidopsis*. Plant Cell 10:2103–2113

Parker JS, Cavell AC, Dolan L, Roberts K, Gierson CS (2000) Genetic interactions during root hair morphogenesis in *Arabidopsis*. Plant Cell 124:1961–1974

Pauwels L, Inzé D, Goossens A (2009) Jasmonate-inducible gene: what does it mean? Trends Plant Sci 14:87–91

Person RL, Stevens LJ (2000) Evidence for the uptake of nonessential ions and essential nutrient ions by root hairs and their effect on root hair development. In: Ridge RW, Emons AMC (eds) Root hairs: cell and molecular biology. Springer, Tokyo, pp 179–195

Pitts RJ, Cernac A, Estelle M (1998) Auxin and ethylene promote root hair elongation in *Arabidopsis*. Plant J 16:553–560

Pré M, Atallah M, Champion A, De Vos M, Pieterse CMJ, Memelink J (2008) The AP2/ERF domain transcription factor ORA59 integrates jasmonic acid and ethylene signals in plant defense. Plant Physiol 147:1347–1357

Rahman A, Hosokawa S, Oono Y, Amakawa T, Goto N, Tsurumi S (2002) Auxin and ethylene response interactions during *Arabidopsis* root hair development dissected by auxin influx modulators. Plant Physiol 130:1909–1917

Robinson D, Rorison IH (1987) Root hairs and plant growth at low nitrogen availabilities. New Phytol 107:681–693

Rojo E, Leon J, Sanchez-Serrano JJ (1999) Cross-talk between wound signalling pathways determines local versus systemic gene expression in *Arabidopsis thaliana*. Plant J 20:135–142

Romera FJ, Alcántara E, De la Guardia MD (1999) Ethylene production by Fe-deficient roots and its involvement in the regulation of Fe-deficiency stress responses by strategy I plants. Ann Bot 83:51–55

Ryan E, Grierson CS, Cavell A, Steer M, Dolan L (1998) TIP1 is required for both tip growth and non-tip growth in *Arabidopsis*. New Phytol 138:49–58

Ryan E, Steer M, Dolan L (2001) Cell biology and genetics of root hair formation in *Arabidopsis thaliana*. Protoplasma 215:140–149

Schiefelbein J, Galway M, Masucci J, Ford S (1993) Pollen-tube and root-hair tip growth is disrupted in a mutant of *Arabidopsis thaliana*. Plant Physiol 103:979–985

Schmidt W, Tittel J, Schilora A (2000) Role of hormones in the induction of iron deficiency responses in *Arabidopsis* root. Plant Physiol 122:1109–1118

Tanimoto M, Roberts K, Dolan L (1995) Ethylene is a positive regulator of root hair development in *Arabidopsis thaliana*. Plant J 8:943–948

Thole JM, Vermeer JEM, Zhang Y, Gadella TWJ Jr, Nielsen E (2008) *Root hair defective 4* encodes a phosphatidylinositol-4-phosphate phosphatase required for proper root hair development in *Arabidopsis thaliana*. Plant Cell 20:381–395

Vitousek PM, Gosz JR, Grier CC, Melillo JM, Reiners WA (1982) A comparative analysis of potential nitrification and nitrate mobility in forest ecosystems. Ecol Monogr 52:155–177

Xu Y, Chang L, Liu D, Narasimhan ML, Raghothama KG, Hasewaga PM, Bressan RA (1994) Plant defence genes are synergistically induced by ethylene and methyl jasmonate. Plant Cell 6:1077–1085

Zhang H, Forde BG (1998) An *Arabidopsis* MADS box gene that controls nutrient-induced changes in root architecture. Science 279:407–409

Zhang H, Forde BG (2000) Regulation of *Arabidopsis* root development by nitrate availability. J Exp Bot 51:51–59

Zhang H, Jennings A, Barlow PW, Forde BG (1999) Dual pathways for regulation of root branching by nitrate. Proc Natl Acad Sci USA 96:6529–6534

Zhu CH, Gan LJ, Shen ZG, Xia K (2006) Interaction between jasmonates and ethylene in regulation of root hair development in *Arabidopsis*. J Exp Bot 57:1299–1308